Original Research Paper

Volume - 11 | Issue - 08 | August - 2021 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Nephrology

ASSOCIATION OF ACE GENE POLYMORPHISM IN PATIENTS WITH DIABETIC KIDNEY DISEASE

Dr. Soma Ananth* MD DM(Nephrology), Assistant professor, Dept. of nephrology, GGH Kurnool. *Corresponding Author

Dr. P S L Sowjanya MD DM, Assistant professor, GGH Kurnool.

Dr. Varaprasad MD DM.

ABSTRACT Diabetes is the leading cause of end stage renal disease in many countries. Many studies have suggested that genetic predisposition plays a crucial role in the development of Diabetic nephropathy as all diabetics will not have renal disease. Hence this study was conducted with an aim to determine the role of ACE polymorphism in diabetic nephropathy.

Material And Methods: We conducted a prospective study on 120 participants attending Nephrology department, dividing into three cohorts with 40 in each group during a period of two years (2013-2015). Three cohorts consisted of healthy , Type 2 Diabetes mellitus without Nephropathy and Diabetes mellitus with nephropathy respectively. After obtaining institutional ethical committee approval and consent from participants Genomic DNA was extracted from peripheral blood using spin column genomic DNA extraction kit (Axygen Biosciences USA) and ACE intron 16 gene was amplified by Polymerase chain reaction (MG series Thermo cycler USA).

Results: Out of 120 participants, Mean age(54.8±5.79yrs), duration of diabetes (11.82±1.98yrs), Systolic blood pressure (171.9±16.44mmHg), Diastolic blood pressure (92.32±8.91mmHg), BMI (25.39±1.83) was high in T2DM with DN and was also statistically significant. ACE gene - II genotype(65%) was more in healthy controls and DD genotype (50%) was more in DN. ACE gene polymorphism was significantly seen between DM, DN with control($P < 0.05$), but not between DM and DN($P > 0.05$).

Conclusion: The current study concluded that demographic and clinical parameters were higher in the nephropathy group as compared to the type 2 diabetes mellitus without nephropathy group. Though ACE DD genotype may increase the risk of type 2 diabetes mellitus , a similar association with an increased risk of diabetic nephropathy was not demonstrated in the present study.

KEYWORDS : Angiotensin converting enzyme; gene polymorphism, diabetes mellitus Type 2; diabetic kidney disease.

INTRODUCTION

The prevalence of type 2 DM is increasing all over the world particularly in the developing countries. India has the world's largest number of diabetics and has emerged as a major public health problem in India.(1,2) Diabetic nephropathy (DN) is one of the most common microvascular complication causing renal impairment in type 1 diabetic (T1DM) or type 2 diabetic (T2DM) patients that leads to dialysis or kidney transplantation (3). About 40% of people with diabetes develop nephropathy irrespective of their glycemic status. DN is a clinical syndrome characterized by reduced glomerulus function, persistent albumin excretion(>300mg/24 hours or 200 microgm/min) through urine and gradual loss of renal function (4).One-third of T2DM patients with excellent blood glucose control can develop to DN,while in most patients,even with anti-hypertensive therapy and suboptimal blood glucose control, DN is not noted.(5, 6) Clustering of DN in T2DM families indicates a strong genetic susceptibility for its development and progression (7). Therefore several genetic factors have been studied to unveil the predisposition to the development of DN in T2DM patients. Due to technical advances in DNA sequencing methods, several candidate susceptibility genes of DN have so far been identified and studied extensively (8).

Rigat et al. first reported that the *ACE* gene insertion/deletion (I/D) polymorphism is caused by the insertion or deletion of a 287 bp Alu repeat sequence within intron 16, which results in three genotypes. While the presence of the DD genotype was found to be associated with the highest level of ACE, the enzyme levels were reported to be the lowest in the II genotype. (9)Recently, many researchers have demonstrated that the *ACE* I/D polymorphism is related to diabetic microangiopathy, and that the D allele might be a susceptibility factor for patients with DKD.(10-12) The frequency of *ACE* alleles varies within different ethnic groups, which might contribute to the conflicting views on the role of the *ACE* gene I/D polymorphism in the development of DKD.(9,13)The ACE I/D polymorphism directly influences circulating levels of ACE. The II genotype protects against the development of diabetic nephropathy.The DD genotype predicts poor renal response to RAAS inhibitors.

The main aim of the present study was to study the association of ACE gene polymorphism in patients with diabetic kidney disease. There have been conflicting reports from various ethnic groups regarding the effect of ACE gene polymorphism on development of diabetic nephropathy. In this context clearly more scope for further studies exist to establish if any association exists between ACE I/D polymorphism and diabetic nephropathy.

MATERIALS AND METHODS

To study the association of ACE gene polymorphism and diabetic nephropathy, a prospective study was undertaken for two years duration (2013-2015) in patients with diabetic kidney disease attending the department of Nephrology.The study was undertaken after obtaining approval from ethics committee of the institution. 120 participants in three cohorts comprising type 2 diabetes without established nephropathy, with nephropathy and normal subjects with 40 in each were included in the study.

Inclusion Criteria:

1) All type 2 diabetes mellitus patients of at least 10 years duration since the time of initial diagnosis.
2) Patients with 24 hr protein of more than 500mg/day.
3) Presence of diabetic retinopathy diagnosed by fundus examination by a qualified ophthalmologist after a mydriatic test were included.

Exclusion Criteria:

1) Type 1 diabetes mellitus
2) Gestational diabetes mellitus
3) Any ongoing sepsis
4) Poorly controlled / accelerated hypertension
5) Congestive heart failure
6) Patients with no evidence of diabetic retinopathy
7) Patients with rapidly declining renal function
8) With active urine sediment
9) Paediatric patients
10) Patients with sudden onset/ progression to massive proteinuria.

Healthy subjects were screened to exclude the presence of type 2 DM using standard criteria.(14) Presence of hypertension was assessed using JNC VIII criteria. Informed consent of the subjects was taken after explaining the procedure to the subjects in their vernacular language. 2ml of blood was collected in EDTA tubes and sent to the lab for DNA extraction and ACE genotype determination.

Angiotensin Converting Enzyme Insertion/Deletion Polymorphism (Genotypes) Analysis

Genomic DNA was extracted from peripheral blood using spin column genomic DNA extraction kit (Axygen Biosciences USA) and ACE

intron 16 gene was amplified by Polymerase chain reaction (MG series Thermo cycler USA).

For amplification, a flanking primer pair [59] 5'-CTGGAGACCACT CCCATCCTTTCT-3' and 5'-GATGTGGCCA TCACATTC GTCACGAT-3' (synthesized by Bio serve Biotechnology) was used. PCR amplification was performed with a 50µl reaction mixture contains 40 pmol of each primer, 200µmol/L each dNTP , 1.5mmol/L $MgCl_2$, 1 U of thermo stable DNA polymerase (DYNAZYME II Espoo, Finland) and 20 mMol of Tris-HCL (pH 8.8 at 25ºC). PCR cycling conditions were carried out with an initial denaturation step of 5 minutes at 95ºC, followed by 30 cycles of denaturation at 95ºC for 30 seconds, annealing at 58ºC for 1 minute and extension at 72ºC for 2 minutes, followed by final extension for 5 minutes at 72ºC before the storage of sample at 4ºC. PCR products were separated by Agarose gel electrophoresis (GENI Bangalore, India). DNA fragments were stained with ethidium bromide and visualized under UV light (Gel documentation system, Biorad USA). The PCR fragments consist of three genotypes, a 490bp band (II), a 190bp band (DD), and both 490 and 190 bp band (ID). To increase the DD genotyping, PCR amplifications were also performed with insertion specific primer pair [60] 5'- TGGGACCACAGCGCCCGCCACTAC-3' and 5'-TCGCCAGCCCTC CCATGCCCATAA-3' for each sample which had the DD genotype to avoid mistyping of ID heterozygotes as D homozygotes. PCR cycling conditions were carried out with an initial denaturation step of 1 minutes at 95ºC,followed by 30 cycles of denaturation at 95ºC for 30 seconds, annealing at 67ºC for 45 seconds and extension at 72ºC for 2 minutes. The PCR product shows a 335 bp band for II allele and no band for DD genotype.

Statistical Analysis

The data was analyzed using the SPSS software. ACE genotype polymorphism frequencies were compared across groups using the one-way ANOVA test, post hoc multiple comparisons test. Chi Square (χ^2) cross tabulation statistics was used to test for difference between frequency data. A p value of < 0.05 was considered significant.

RESULTS

The present study was conducted with the aim to understand the role of ACE gene polymorphism in occurrence of Diabetic nephropathy on 120 subjects in three cohorts with 40 in each.

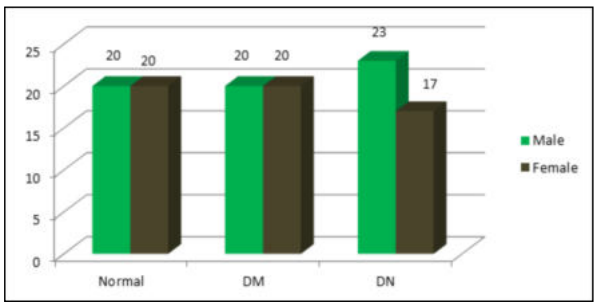


Fig -1 Sex Distribution

The number of males and females were equal in both the normal group and with diabetes (DM) group without nephropathy. While in the diabetic nephropathy group there were 23(57.5%) males and 17(42.5%) females as shown in Fig-1.

Table-1 Demographic And Clinical Association Between The Cohorts.

	Control	DM	DN	F	P
Age	50.15±2.59	54.95±5.22	54.8±5.79	13.24	<0.0001
Duration of DM	0	11.75±1.41	11.82±1.98	940.3	<0.0001
Systolic BP	119.05±7.26	148.45±5.51	171.9±16.44	238.1	<0.0001
Diastolic BP	76.77±5.46	88.7±4.42	92.32±8.91	61.71	<0.0001
BMI	22.75±0.83	24.73±0.9	25.39±1.83	46.72	<0.0001

The mean age of normal subjects is 50.15 ± 2.59 , DM was 54.95 ± 5.22 and diabetes with nephropathy group was 54.80 ± 5.79.The mean age, duration of the disease, SBP,DBP and BMI was more in diabetic nephropathy group. Statistically significant association between demographic and clinical variables with ANOVA test was seen between the groups.(Table 1)

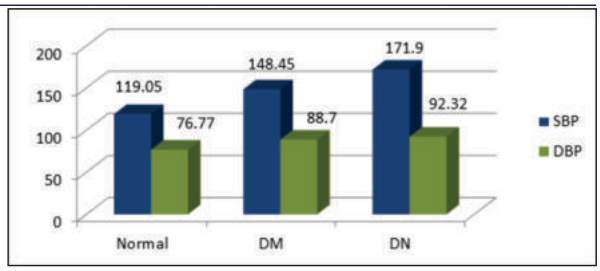
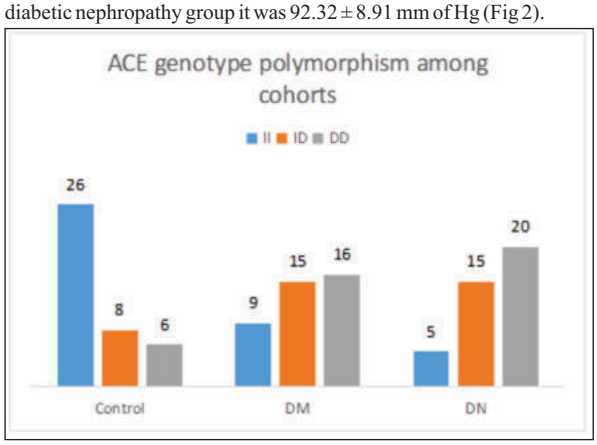


Fig - 2 Distribution of blood pressures in the three cohorts

The blood pressure among the type 2 DM with DN groups was higher as compared to the T2DM without DN whose was higher to control group. The mean systolic BP in the control group was 119.05 ± 7.26 mm of Hg as compared to148.45 ± 5.51 in type 2 DM group. In nephropathy group it was 171.90 ± 16.44mm of Hg. The mean diastolic blood pressure in the control group was 76.77 ± 5.46 mm of Hg while in type 2 DM group it was 88.70 ± 4.42 mm of Hg and in the diabetic nephropathy group it was 92.32 ± 8.91 mm of Hg (Fig 2).



$\chi 2P$ value: Control Vs DM 14.93,<0.05*; DM Vs DN 1.58,0.45; DN Vs Control 23.89, <0.0001*

Fig - 3 ACE gene genotypes among cohorts

Analysis of the results showed that the ACE gene distribution in the control group has II genotype in 26 (65%) , while DD genotype in 16 (40%) in DN-(T2DM without Nephropathy) group and 20(50%) has DD genotype in DN+ (T2DM with DN) (Fig-3).

Table-2 Showing P Values (chi-square Test) In The 3 Cohorts

ACE gene genotype	Normal (%)	DM (%)	DN (%)	DM Vs DN	Control Vs DM	Control Vs DN
II	26 (65)	9 (22.5)	5 (12.5)	P > 0.05	P < 0.001*	P < 0.001*
ID	8 (20)	15(37.5)	15 (37.5)	P - 1	P > 0.05	P > 0.05
DD	6 (15)	16 (40)	20 (50)	P > 0.05	P < 0.05*	P < 0.001*

The frequency of DD genotype was significantly higher in DM subjects as compared to controls (p<0.001) .The frequency of DD genotype was significantly higher in DN group as compared to controls (p < 0.001).The frequency of DD genotype was higher in the diabetic nephropathy as compared with the diabetes without nephropathy group. However it was not statistically significant (P>0.05) and was shown in table - 4. Similarly statistically significant II genotype was higher in DM with or without DN compared to Control group.

Table - 3 Post Hoc Tests - Multiple Comparisons

Dependent Variable	(I) Subjects	(J) Subjects	Mean Difference (I - J)	Sig p.
Systolic BP	Normal	DM	-29.40000*	.000
		DN	-52.85000*	.000
	DM	Normal	29.40000*	.000
		DN	-23.45000*	.000
	DN	Normal	52.85000*	.000

		DM	23.45000*	.000
Diastolic BP	Normal	DM	-11.92500*	.000
		DN	-15.55000*	.000
	DM	Normal	11.92500*	.000
		DN	-3.62500*	.039
	DN	Normal	15.55000*	.000
		DM	3.62500*	.039
BMI	Normal	DM	-1.98150*	.000
		DN	-2.64350*	.000
	DM	Normal	1.98150*	.000
		DN	-.66200	.057*
	DN	Normal	2.64350*	.000
		DM	.66200	.057*

*Statistically significant

Further analysis by Post hoc Multiple comparisons revealed a mean difference of 29.4 mm of Hg between the type 2 diabetes and the control group (p=0.000) while it was 52.85 mm of Hg between the nephropathy group and the controls (p=0.000). There was also a significant difference of systolic blood pressure of 23.45 mm of Hg between the diabetic nephropathy and the type 2 DM group (p=0.000).The diastolic blood pressure among the three groups revealed a difference of 11.92 mm of Hg between the type 2 DM group and control group (p=0.000) as compared to mean difference of 15.55 mm of Hg between the diabetic nephropathy and control group (p=0.000). The mean difference of 3.62 mm of Hg between the diabetic nephropathy and DM group (p=0.039). The mean difference in BMI was 1.98 between the DM group as compared to control group (p=0.000) while it was 2.64 in diabetic nephropathy as compared with control subjects (p=0.000). The mean difference between DM and DN was 0.66 which did not attain statistical significance.(Table - 3)

DISCUSSION

T2DM patients are more prone for chronic kidney disease leading to end stage renal disease. Predicting renal failure in diabetic patients is very complicated. The duration of diabetes, status of glycaemic control and blood pressure are not enough to predict nephropathy. The genetic and environmental interactions has crucial role in progression of renal disease in T2DM. Hence evaluating the ACE I/D polymorphism in diabetic nephropathy can be a reliable tool to identify diabetic patients at risk.

The present study aimed to investigate the role of ACE gene polymorphism in the development of diabetic nephropathy. Statistically significant association between three cohort groups(healthy, T2DM without nephropathy, with nephropathy respectively) regarding age, duration of Diabetes, blood pressure, BMI was seen in our study. All these characteristics were more in T2DM with Diabetes nephropathy according to our study.

Mean age, duration of diabetes, BMI was comparatively high in T2DM with DN compared to healthy controls and T2DM without DN in other studies done in Asia. Similar association between BP and DN was seen in other studies.(15-20) These results were similar to the findings of our study. But few studies even confirmed that there were no significant differences of BMI, duration of diabetes,SBP and DBP between healthy and DN groups.(19)

This study demonstrated that frequency of ACE - DD genotype frequency is higher in DM and DN when compared to normal subjects. The frequency of ID polymorphism is similar in both DM and DN groups. The frequency of DD polymorphism is high in DN subjects when compared to DM subjects however it was not statistically significant (p>0.05). II genotype was protective as they were more in normal subjects and DD genotype was more in DN group indicating that it is a better evaluating tool of renal impairment.

DD genotype was more expressed in DN groups compared to healthy subjects in other studies(18) and statistically associated too(19,) which was similar to our study. Though DD genotype was more in DN, statistical association was not seen in other studies.(20,21) Contrary to our study findings many studies stated that ACE gene polymorphism has no role in development or progression to renal disease.(17) Mizuri et al in 111 Japanese type 2 DN subjects, showed that II genotype was associated with a decreased risk of DN.(22) Similarly in the present study too the frequency of distribution of II genotype is more in normal subjects when compared with DM and DN subjects.

In the present study association between T2DM with and without Nephropathy was not seen. Though association between T2DM with/without DN and healthy controls was statistically significant.Lakkakula BVSK et al in their meta analysis involving around 45 articles (47 studies) with 6124 patients of DN and 2492 T2DM patients (controls) concluded that the ACE I/D polymorphism is correlated with an increased risk of DN in patients with T2DM and the D allele of ACE I/D was a susceptible factor(23).Many caucasian and Asian studies especially in India has confirmed the association of ACE gene polymorphism and Diabetic nephropathy, though many studies were inconclusive.

However present study group was small. Further studies are required with inclusion of more number of patients to find the association of ACE gene polymorphisms in the development of DN. Follow-up studies of the patients could give more evidence to the relationship of the genotypes with the severity and the rate of decline of kidney function.

CONCLUSION

Diabetic kidney disease contributes to Chronic kidney disease, ultimately to end stage renal disease. The present study concludes that mean age, duration of diabetes, BMI, BP elevated in T2DM with Diabetic nephropathy and statistically significant association seen except for BMI. Though we confirm that ACE gene polymorphism is associated with Diabetes, significant association with nephropathy is not demonstrated.

REFERENCES

1) Basoum R S; Chronic Kidney Disease in the Developing world; Engl J Med 2006; 354 ;997-99.
2) Sahay B K;The API-ICP guideline on Diabetes 2007:JAPI 2007:55:1-50.
3) Fineberg D, Jandeleit-Dahm KA, Cooper ME. Diabetic nephropathy: diagnosis and treatment. Nat Rev Endocrinol. 2013;9(12):713-23.
4) Lim AK. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361-81.
5) Rahimi Z. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. Journal of Nephropathology. 2012;1:143–51.
6) Shin YS, Baek SH, Chang KY, Park CW, Yang CW, Jin DC. Relations between eNOS Glu298Asp polymorphism and progression of diabetic nephropathy. Diabetes Research and Clinical Practice. 2004;65:257–65.
7) Arar NH, Hazuda HP, Plaetke R, Sartorio V, Arar MY, Abboud HE. Familial Clustering of Diabetic Nephropathy: Perceptions and Risk Recognition Among Mexican-American Patients With a Family History of Diabetes. Diabetes Spectr. 2003;16(3):136-42.
8) Lakkakula S, Gupta P, Kumar Verma H, Lakkakula BVKS. Association between PPARG Pro12Ala polymorphism and diabetic nephropathy risk; an updated metaanalysis of 27 studies. J Renal Endocrinol. 2018;4(1):11e.
9) Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86(4): 1343–1346.
10) Ramanathan G, Elumalai R, Periyasamy S, Lakkakula B. Role of renin-angiotensin-aldosterone system gene polymorphisms and hypertension-induced end-stage renal disease in autosomal dominant polycystic kidney disease. Iran J Kidney Dis. 2014;8(4):265-77.
11) Xie X, Liu Y, Perkovic V, Li X, Ninomiya T, Hou W, et al. Renin-Angiotensin System Inhibitors and Kidney and Cardiovascular Outcomes in Patients With CKD: A Bayesian Network Meta-analysis of Randomized Clinical Trials. Am J Kidney Dis. 2016;67(5):728-41.
12) Ramanathan G, Ghosh S, Elumalai R, Periyasamy S, Lakkakula BV. Influence of angiotensin converting enzyme (ACE) gene rs4362 polymorphism on the progression of kidney failure in patients with autosomal dominant polycystic kidney disease (ADPKD). Indian J Med Res. 2016;143(6):748-55.
13) Alhenc-Gelas F, Baussant T, Hubert C, Soubrier F, Corvol P. The angiotensin converting enzyme in the kidney. J Hypertens Suppl. 1989;7(7):S9-13.
14) J. Larry Jameson, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, Dan L. Longo, Joseph Loscalzo. Diabetes mellitus, Harrison's Principles of Internal Medicine. Mc Graw Hill New York, 20e, 2152-4.
15) Mahwish UN, Ponnaluri KC, Heera B, et al. Link between ACE I/D Gene Polymorphism and Dyslipidemia in Diabetic Nephropathy: A Case-control Study from Hyderabad, India. *Indian J Nephrol*. 2020;30(2):77-84. doi:10.4103/ijn.IJN_244_18.
16) Jeffers BW, Estachio OR, Raynolds MV, Schrier RW : Angiotensin converting enzyme polymorphism in non-insulin dependent Diabetes Mellitus and its relationship with Diabetic Nephropathy : Kid int 1997:52:473-477.
17) Schimdt S, Schone N ,Ritz E and the diabetic nephropathy study group: Association of ACE polymorphism and diabetic nephropathy? Kidney int :1995;47:1176-1181.
18) V. V. S. Naresh, A. L. K. Reddy, G. Sivaramakrishna, P. V. G. K. Sharma, R. V. Vardhan and V. Siva Kumar: Angiotensin converting enzyme gene polymorphism in type II diabetics with nephropathy: Indian J Nephrol, 2009 October; 19(4): 145–148.
19) Vijay V , Yanqing Z , Karthik B , S Dunn , C Snehalatha, Ambady R , Muthu J , Kumar S. Association between ACE Gene Polymorphism and Diabetic Nephropathy in South Indian Patients.*JOP. J. Pancreas (Online) 2001; 2(2):83-87.*
20) Wyawahare M, Neelamegam R, Vilvanathan S, Soundravally R, Das AK, Adithan C. Association of AngiotensinConverting Enzyme Gene Polymorphisms and Nephropathy in Diabetic Patients at a Tertiary Care Centre in South India. Clin Med Insights Endocrinol Diabetes. 2017;10:1-5.
21) Ahluwalia TS, Ahuja M, Rai TS, Kohli HS, Bhansali A, Sud K, et al. ACE variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy. DNA Cell Biol. 2009;28(3):141-50.
22) Mizuiri S, Hemmi H, Inoue A, Yoshikawa H, Tanegashema M, Fushimi T et al: Angiotensin converting enzyme polymorphism and development of Diabetic Nephropathy in NIDDM : Nephron 1995;70: 455-459.
23) Lakkakula BVSK, Khare RL, Verma HK, Pattnaik S. Genetic association of ACE gene I/D polymorphism with the risk of diabetic kidney disease; a meta-analysis. J Nephropathol. 2019;8(4):e44. DOI: 10.15171/jnp.2019.44